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1. PURPOSE

This protocol describes PCR amplification of eukaryotic cDNA plasmid inserts, gel electrophoresis, purification, and storage of PCR products.

2. SCOPE

This procedural format is currently utilized by Human Colon Cancer and Mouse microarray projects under the supervision of John Quackenbush within the Eukaryotic Genomics Dept.

3. MATERIALS

3.1 AmpliTaq® DNA Polymerase with GeneAmp® PCR Reaction Kit (Applied Biosystems; Cat # N808-0156)
-10X PCR Buffer II
-$MgCl_2$ Solution
-Platinum Taq Polymerase
3.2 M13 Forward and Reverse Primers (Life Technologies)
- Forward: 5' GTT TTC CCA GTC ACG ACG TTG 3'
- Reverse: 5' TGA GCG GAT AAC AAT TTC ACA CAG 3'
3.3 dNTP kit (100mM of each dNTP) (Life Technologies; Cat # 10297-018)
3.4 MilliQ water
3.5 MicroAmp® Optical 96-well Reaction Plate (Applied Biosystems; Cat # N801-0560)
3.6 MicroAmp® 96-well Full Plate Cover (Applied Biosystems; Cat # N801-0550)
3.7 Multiscreen® PCR filter plate (Millipore; Cat # MANU3050)
3.8 Cap mat (VWR; Cat # 40002-002)
3.9 Falcon Microtest U-bottom 96 well plate (BD Biosciences; Cat # 353077

4. PROCEDURE

4.1 PCR of cDNA plasmid inserts.

4.1.1 After cDNA clones have been cultured and plasmids isolated (see SOP- M001), a 1:10 dilution is made of the concentrated plasmid isolate (10 µL in 90 µL of MilliQ H_2O).

4.1.2 Prepare a master mix for 100 µL PCR reactions according to the following mixture:

MilliQ water……	7.32 mL
10X PCR Buffer……..……..	1 mL
$MgCl_2$ (25 mM)……..	…….. 1 mL
M13 Forward primer (10µM)…….	200 µL
M13 Reverse primer (10µM)……..	200 µL
dNTP mix (25mM per dNTP)*…...80 µL	
(* 20 µL of each dNTP as 100 mM stock)	
Platinum Taq (5U/µL)…….40 µL	
Total Volume/Plate	9.84 µL

Note: Keep all reagents on ice while preparing the master mix. Add the Platinum Taq Polymerase last taking it directly from the freezer and returning it promptly.

Note: Prior to preparing PCR sterilize work space, wear gloves and always use sterile tips to avoid contamination.

4.1.3 Mix master mix well and decant into a sterile reservoir. Using an automatic multichannel pipettor (8 channel Matrix Impact^2) dispense 96 µL of mix into each well of a MicroAmp® Optical PCR reaction plate.

4.1.4 Add 4 µL of 1:10 diluted plasmid (from step 4.1.1) to each well containing master mix. Mix well with the pipette.

4.1.5 Place a MicroAmp® Full Plate Cover on the PCR plate and centrifuge at 2700 rpm for 1 minute.

4.1.6 Place PCR plates in a thermocyler (MJ Research; PTC-225) and run the following cycling program:

Initial Denaturation……	95°C x 2 min.	
Denaturation……..	95°C x 30 sec.	x 30 cycles
Primer annealing……	52°C x 30 sec.	
Primer extension……	72°C x 2 min.	
	4°C forever	

4.1.7 Spin down the PCR plates and store PCR products at 4°C or frozen at –20°C.

4.2 Purification of PCR product

4.2.1 Spin down PCR reaction plates and then transfer the PCR products (100µL) to a Multiscreen® filter plate and place the filter on a vacuum manifold filitration system (Millipore; Cat # MAVM0960R).

4.2.2 Apply a vacuum pressure of approx. 10-15 inHg (250-380 mmHg) for ten minutes or until plate is dry.

Note: Filter until no more fluid is visible in the well. The filter appears wet (shiny) even when dry so do not use the appearance of the filter as a guide. Also check all of the wells in the plate before removing from vacuum; some wells filter more slowly than others.

4.2.3 Remove plate from manifold filtration system and add 100 µL of MilliQ water to each well.

4.2.4 Place filter plate on a shaker and shake vigorously for 20 minutes to resuspend the DNA.

4.2.5 Manually pipet the purified PCR product to a new Falcon U-bottom 96 well plate.

4.3 Storage of PCR products

4.3.1 Seal PCR storage plates with a plastic cap mat or adhesive foil lid and store at –20°C until needed for making microarray printing plates.

4.3.2 For long term storage after filtration aliquot equal volumes of purified PCR product into multiple plates. Store one plate with a cap mat at –20°C (for short term use) and dry down the remaining plates in a speed vac and store in a dry cabinet.

4.3.3 Resuspend dried PCR product in MilliQ water as needed to make microarray printing plates.